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TITLE: DEVELOPMENT OF ULTRA LONG DURATION LOCAL ANESTHETIC AGENTS IN A RAT MODEL

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13. ABSTRACT *(Maximum 200 words)*

1. Two abstracts were submitted to the American Society of Anesthesiology and will be presented at this national meeting in Oct 94. Two abstracts and one demonstration booth were presented at the Regional Anesthesia Society in Chicago April 94. One paper was published and one manuscript is in press.

2. These projects were used for resident training projects for Doctors Kuzma, Calkins and Burnham.

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13. Abstract (continued)

3. We demonatrated that lecithin coated bupivacaine microcrystals provided reversible ultra long duration local anesthesia in a rat model. The microencapsulated depot produced a local anesthetic effect for 44 hours compared to 5 hours for control animals receiving neat bupivacaine.

4. We showed that 10% microencapsulated bupivacaine microcrystals were not more tissue toxic than neat 0.75% bupivacaine.

5. We showed that 10% microencapsulated bupivacaine microcrystals did not cause spinal cord toxicity when injected intrathecally.

6. As we are approaching an IND submission for topical use of microencapsulated local anesthetics/analgesics our laboratory was moved to the Dept. of Clinical Pharmacology, USUHS where it would be possible to perform GLP work if necessary.

7. A pre IND meeting is planned for the Fall of 1994 to discuss parenteral products. A topical IND is already being compiled.

8. A patent was submitted for a new microencapsulation formulation with Doctors Haynes, Boedeker and Kline as inventors.

PI: Mark Kline, MD

TITLE: TISSUE TOXICITY OF LECITHIN-COATED BUPIVACAINE MICROCRYSTALS AFTER INTRADERMAL INFILTRATION

Introduction: Lecithin-coated bupivacaine microcrystals have been shown to provide ultra long duration local anesthesia (44 hours) when injected subcutaneously in the rat tail.[1] The ultra long duration presumably results from a slow, continuous release of bupivacaine from the preparation. Prior to utilizing this agent for treatment of postoperative or chronic pain, lack of tissue toxicity must be established. The present study evaluated the toxicity of lecithin-coated bupivacaine microcrystals when injected intradermally in the rat.

Methods: 0.1 cc of each test agent was placed intradermally via a 23-gauge needle on the shaved mid-back skin of 18 anesthetized rats. Agents tested were 10% lecithin-coated bupivacaine microcrystals, 0.75% bupivacaine solution, lecithin membranes without bupivacaine, and 5% dextrose in water (D5W). Rats were sacrificed in groups of 6 at 24 hours, 3 days, and 7 days after injection, with full thickness skin biopsies taken from each injection site as well as a non-injected control site. After formalin fixation and H and E staining, 5 mm long skin specimens were evaluated for inflammatory reaction using a previously described method.[2,3] The degree of inflammation was graded by neutrophilic accumulation, which was scored as follows: 1= 6 to 40 neutrophils (PMNs) present; 2 = more than 40 PMNs present, with moderate number of focal collections and/or few scattered; 3 = more than 40 PMNs present with extensive foci and few scattered; and 4 = extensive foci and marked numbers of scattered PMNs. A total score for each site was obtained by adding scores for superficial and deep inflammation. Mean scores were compared using the Wilcoxon signed rank test, with $p < 0.05$ considered statistically significant.

Results: Comparison of the mean inflammation scores revealed no statistically significant difference at any time point in the level of tissue inflammation caused by 10% lecithin-coated bupivacaine microcrystals, 0.75% bupivacaine solution, and lecithin membranes without bupivacaine. All three solutions produced a greater inflammatory response (statistically significant) than that observed with D5W or non-injected control at 24 hours. At 3 days and 7 days, however, there was no statistically significant difference in inflammatory response between any of the test agent groups and the non-injected control group.

Discussion: At no time during our study did 10% microencapsulated bupivacaine microcrystals produce a statistically significant difference in inflammatory response when compared to 0.75% bupivacaine solution. Microcrystal technology allowed delivery of a depot of bupivacaine at thirteen times the concentration of the clinically used 0.75%

TITLE: MICROCRYSTALLINE BUPIVACAINE COATED WITH LECITHIN PRODUCES ULTRA LONG DURATION LOCAL ANESTHESIA IN THE RAT TAIL

AUTHORS: PJ Kuzma, MD, MD Kline,MD, BH Boedeker,MD, DH Haynes PhD

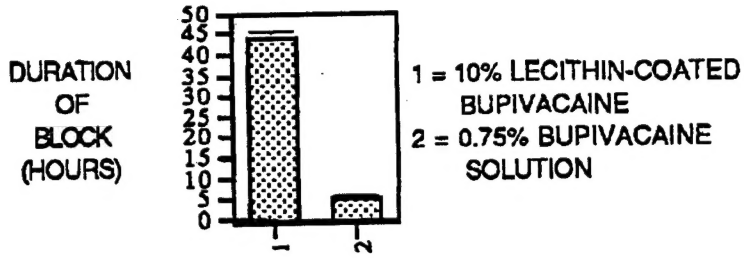
AFFILIATION: Walter Reed Army Institute of Research, Washington, D.C. 20307

Introduction: Ultra long duration local anesthetics would be clinically useful for treating acute, chronic, and cancer pain. Lecithin-coated tetracaine microcrystals have been shown to produce a rat tail block of 43 hours duration (1) and to be non-toxic when used intradermally or when applied directly to the sciatic nerve (2,3). The present study evaluated the efficacy of bupivacaine microcrystals coated with lecithin in producing ultra long duration local anesthesia in the rat tail.

Methods: The presence of local anesthesia was evaluated by measuring the tail-flick response to radiant heat stimuli (4). Ramping radiant heat stimuli was delivered to differing tail skin sites, and stimulus intensity was adjusted to elicit tail withdrawals at a constant latency of between 3 to 5 seconds (mean 4.1). A subcutaneous ring block was instilled in the mid tail region using 0.3 cc of test agent. The tail was subjected to the same intensity stimulus and return of latency to within 120% of baseline was considered a positive response and equated with lack of local anesthesia. If the duration of stimuli exceeded 200% of the baseline latency without a tail-flick response, the stimulus was stopped, and this was considered a negative response and equated with adequate local anesthesia to the area tested. Agents evaluated included 10% lecithin-coated bupivacaine microcrystals (group 1)and 0.75% bupivacaine (group 2). Statistical correlation was measured using the paired, two tailed t-test with p value < 0.02 considered statistically significant.

Results: Animals in Group 1 (10% microencapsulated bupivacaine) showed a tail block lasting 44.2 ± 0.4 hours (expressed as SEM with $n = 5$). Group 2 (0.75% bupivacaine) had a tail block lasting 5.0 ± 0.8 hours ($n = 4$). Onset of block in Groups 1 and 2 was within 5 minutes.

Discussion: These data suggest that lecithin-coated bupivacaine microcrystals provide reversible ultra long duration local anesthesia. Microcrystal technology allows for the delivery of high concentrations of drug. Slow dissolution of the microcrystal and/or a barrier effect of the lecithin membrane may account for the sustained, reversible effect of the bupivacaine microcrystals. Further studies will evaluate the pharmacokinetics of the microencapsulated agent, but initial data suggest the drug is released in a sustained fashion from the microencapsulated depot to produce an ultra long acting local anesthetic effect of 44 hours duration.



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1. Anesthesiology **77**: A799, 1992.
2. Anesthesiology **77**: A800, 1992.
3. Anesthesiology **79**: A825, 1993.
4. Animal Pain, Churchill Livingstone, 97-99.